Two Dimension (2D) elasticity maps of coagulation of blood using SuperSonic Shearwave Imaging

Miguel Bernal, Jean-Luc Gennisson, Patrice Flaud, Mickaël Tanter

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M. Bernal, J.-L. Gennisson, P. Flaud and M. Tanter

Institut Langevin - Ondes et Images, 10, rue Vauquelin, ESPCI ParisTech, CNRS UMR7587, INSERM U979, 75005 Paris, France
miguel.bernal@espci.fr

Introduction. Deep venous thrombosis affects millions of people worldwide. A fatal complication occurs when the thrombi detach and creating a pulmonary embolism. The diagnosis and treatment of DVT depends on clot's age. It has been shown that the elasticity of thrombi is closely related to its age. Methods. Blood from 9 live pigs was collected and anticoagulated using EDTA. Coagulation was initiated using calcium ions in saline solution. SuperSonic shear wave imaging was used to generate shear waves using 100 µs tonebursts of 8 MHz at 3 different location in the lateral direction. Tracking of the shear waves was done by ultrafast imaging. Results. Two dimensional (2D) map of elasticity were obtained by calculating the speed of shear wave propagation. Elasticity varied with time from 50 Pa at coagulation to 1300 Pa at 120 minutes. Ejection of the serum from the clot showed to decreased the elasticity of the clot next to liquid pool, corresponding to the detachment of the clot from the beaker wall. Conclusion. SuperSonic imaging proved to be useful mapping the elasticity of clots in 2D. It allows the visualization of the heterogeneity of mechanical properties of thrombi and has potential use in predicting thrombi breakage.

1 Introduction

Deep venous thrombosis (DVT) is a disease that affects millions of people worldwide each year. About 30 to 50 % of the patients that suffer of DVT will develop pulmonary embolism, a complication that happens when the thrombi breaks off and blocks the pulmonary arteries. Between 60.000 to 200.000 patients die of this complication each year in the United States alone[1], and around 370.000 in Europe [2].

It has been shown that knowing the age of the thrombus helps with the risk stratification and therapy planning. The mayor risks occurs when part of the clot breaks or the whole clot detaches from the venous walls, it is important to be able to predict this event. It has been shown before that "acute" thrombi are more prone to detach than "mature" and stable clots [3], [4]. The age of the thrombi and the mechanical properties are closely related as was shown by Emelianov *et al* [5]. Gennisson in 2006 and Schmitt in 2011, used mechanical vibrators to generate shear waves in coagulated blood. By measuring the speed and the attenuation of the shear waves, they were able to recover the viscoelastic properties of coagulated blood.

In this work we continue to explore shear waves and they use in characterizing the mechanical properties of thrombi. Here we used SuperSonic shear wave imaging to generate two dimension (2D) maps of shear elasticity during the coagulation process and show its use in the study of the clots heterogeneity and their risk of detachment.

2 Methods

2.1 Blood samples

Nine porcine blood samples were used in the present study. The blood was collected from a veterinary hospital in accordance with The Fair and Responsible Animal Research Agreement. After getting the samples, the blood was immediately anticoagulated using EDTA (Ethylenediaminetetraacetic acid, number E-9884, Sigma Chemical, St. Louis, MO, USA) diluted in a saline solution at a concentration of 0.83%. This solution was added to the containers prior to the collection according with the amount of blood expected in order to achieve a concentration of 2mg of EDTA per ml of blood. The blood samples were either used the same day (2 hours after collection) or the next day (after being refrigerated overnight at 4 ºC). used. Coagulation started using a calcium solution at 5%. Four ml were mixed with the blood samples (100 ml each) for each of the experiments.

2.2 SuperSonic Imaging

SuperSonic Imaging was used to measure the elastic properties of the blood samples. The technique combines ultrasound radiation force to generate a quasi plane shear wave with ultrafast imaging to track the propagation of such wave[6]. The set up for the blood experiments is presented in Figure 1. The lateral localization of the excitation pushes was at elements 64, 128 and 192 (shown as white arrows in Figures 3B and 4B). In the depth (z) direction the pushes were generated at 10, 15, 20 and 25 mm, in order to create the "quasi" plane wave. The time between pushes at different depths was faster than the speed of the shear wave (supersonic). Each push was generated by a toneburst with a length of 100 µs at 8MHz. The propagation of the shear wave was measured by ultrafast ultrasound imaging. Using the same transducer, a plane ultrasound wave (different from the plane shear wave) was used to image the field of interest at a frequency of 2kHz. All the backscatter radio frequencies were recorded in each element of the array and stored in memory. The beamforming process was done in the receiving mode and post acquisition.

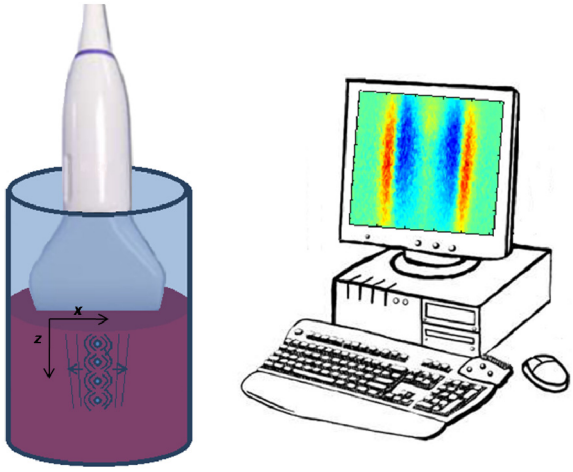

Figure 1: Experimental setup for blood coagulation experiments.

The visualization of the shear waves was done by correlation algorithms between successive speckle images reconstructed from the RF data. The acquisition (ultrasound radiation sequence and ultrafast imaging sequence) lasted 50 µs and was done every 60 seconds for 120 minutes.

The shear wave elasticity maps were estimated using a correlation algorithm to calculate the time of flight of the shear waves and from there their velocities [6]. In this paper

we assume that once the blood coagulates the medium is mostly elastic and the viscosity was neglected. Therefore, using Eq. 1 we calculated the shear modulus (μ).

$$\mu = V_s^2 \cdot \rho \qquad (1)$$

where *Vs* is the shear wave speed, and ρ the blood density (1060 kg/m^3)[7].

3 Results

Figure 2 shows the progression of the coagulation process. Panel A to D show the 2D elasticity maps at 0 minutes of coagulation, 10, 50 and 120 (end of the experiment). Similarly, Panels E to F show the corresponding Bmode images.

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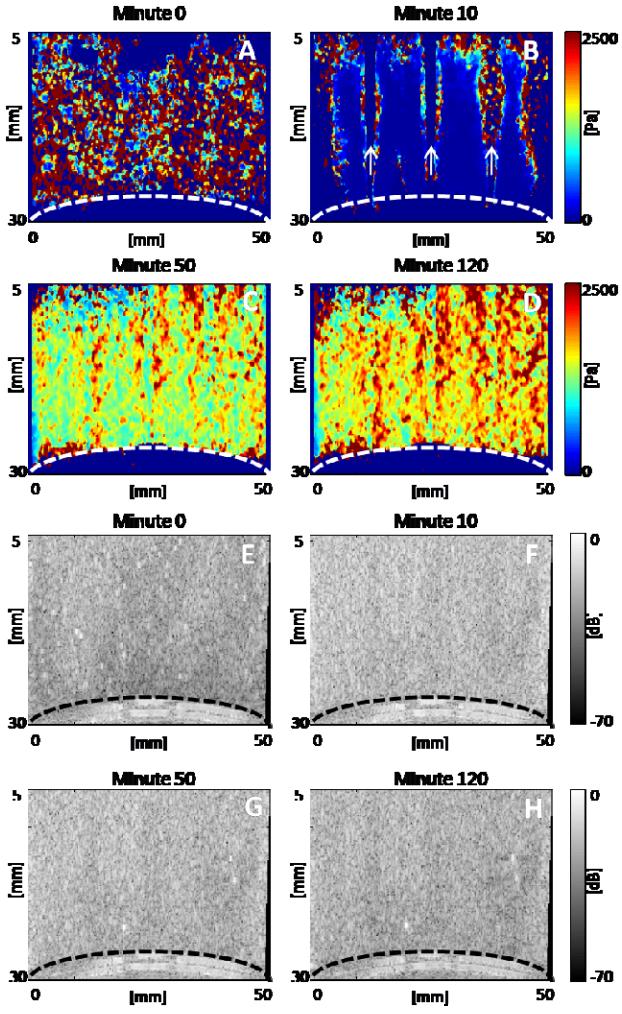

Figure 2: Two dimension elasticity maps and Bmode images along the coagulation process. The white (in the elasticity maps) and the black (in the Bmode images) represent the bottom of the plastic beakers where the blood samples were held.

Figure 3 shows the effect of syneresis. Syneresis is a phenomenon that was first described in 1923 by Pickering and Hewitt in 1923. This phenomenon describes the expulsion of water from a clot as this one contracts. In this figure, in Panels C and D a pool of liquid is visible in Bmode images at the bottom of the transducer. The two time points (30 and 120 minutes) show the grow of the liquid pool which cannot escape to the surface. The continues white line in Panels A and B and yellow line in Panels C and D show the contour of the liquid pool. Panels A and B, shows the 2D elasticity map at minute 20 and minute 120. The red and blue boxes in the images correspond to 2 different ROI's. The blue one is an ROI far away from the syneresis process while the red one is adjacent to it.

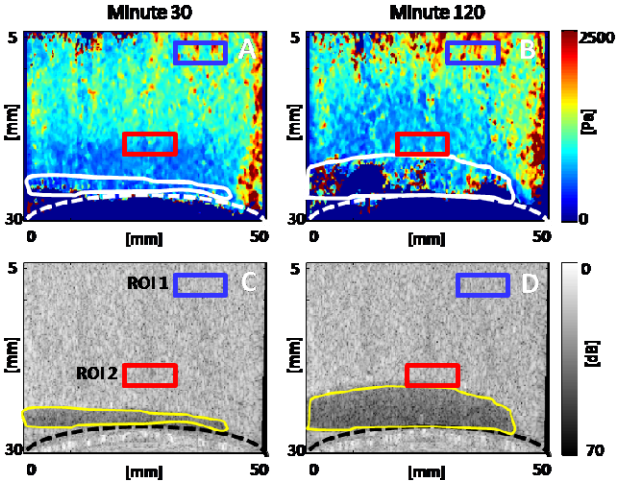

Figure 3: Syneresis phenomenon as seen in 2D elasticity map as well as Bmode image at 2 different time points.

Figure 4 shows the progression of the median elasticity values within the 2 ROIs and the whole 2D field with time.

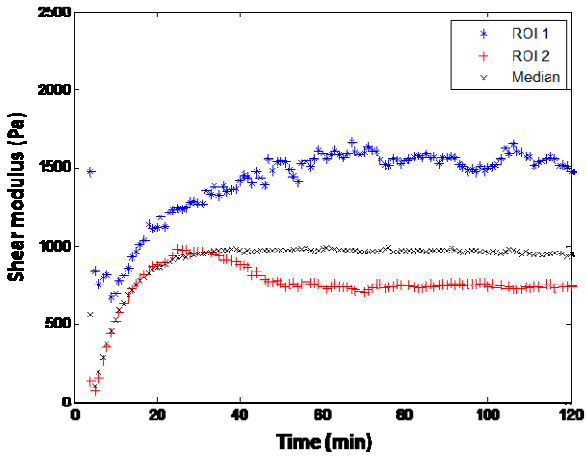

Figure 4: Syneresis phenomenon as seen in 2D elasticity map as well as Bmode image at 2 different time points.

Table 1 shows values for 3 experiments done at the same time on blood samples from the same pig. These experiments were to evaluate the repeatability of the coagulation and the measurement processes.

Table 1: Repeatability results.

Sample number	Hematocrit (%)	t_0 *(min)*	$\mu_0 \pm std$ *(Pa)*	$\mu_p \pm std$ *(Pa)*
1-1	24	10	59.2± 19.1	1463.8± 231.1
1-2	24	10	72.3± 16.7	1341.1± 246.1
1-3	24	10	53.3± 18.3	1373.7± 315.4

where μ_0 is the lowest shear elasticity measured, meaning that shear waves are supported by the medium, due to the change from a liquid to a soft solid state. The value t_0 represents the time at which μ_0 is observed; and μ_p is the plateau shear elasticity (here measured at the median value of the last 10 minutes of the experiment.

4 Discussion

During the experiments, other than the visualization of the syneresis phenomenon in the Bmode images, there were no significant changes in the scatter distribution or the intensity with time. On the contrary, the changes in the 2D elasticity maps were significant with time. It starts with noise due to the absence of shear waves in liquids until the blood coagulates enough for shear waves to propagate. This phenomenon can be observed in Figure 2B, where the location of the three pushes (white arrows) can be seen next to very soft areas. At this stage, the softness of the thrombus is such that the shear waves do not propagate more that few millimeters and the 3 pushes are not enough to fill the 2D space with shear waves. Nevertheless, in the following acquisition (minute 11 for Figure 2) the elasticity maps are complete (data not shown). In panels 2B, 2C and 2D, the progression in the elasticity change is quite notorious with medians of 72, 1418 Pa and 1820 Pa at minutes 10, 50 and 120, respectively. The standard deviations also increased from 16 to 313 and to 439 Pa, respectively. This findings suggest that the visualization of the coagulation process in a 2D fashion can be highly advantageous since the homogeneity of the thrombus seems to change with time.

As mention before, syneresis is the expulsion of serum from the thrombus as this one contracts [8]. This phenomenon was observed in a number of our experiments in the Bmode images, either at the top or the bottom of the beaker. In some cases it was not possible to visualize in the Bmode even though expulsion of the serum was visible on top of the thrombi. In Figure 3, bottom row (Bmode images), is possible to see the syneresis phenomenon at two different time points, and how the amount of serum expulsed increases with time. In this case the liquid pocket is localized toward the bottom of the beaker. An interesting observation is the effect that this liquid pocket has on the elasticity of the thrombi in its vicinity. In the top row of Figure 3 (elasticity maps), it is possible to differentiate two regions, soft and a hard areas. The soft area appears with the creation of the liquid pocket and grows as the amount of liquid increases. An interesting observation was that the thrombi detached from the beaker where the syneresis process happened, meaning where the elasticity decreased to its lowest. This seems to suggest that detachment or rupture of the thrombi could be predicted from the analysis of the 2D map elasticity. This phenomenon was reproducible when the syneresis was observed in the Bmode (54 % of the experiments).

Evaluation of these different regions in time showed interesting results. The coagulation in both regions happens at the same time and the elasticity starts to increase in a similar fashion (Fig. 4). Nevertheless, around minute 13 when the pocket of liquid starts to form, the curves describing the median of the two regions and that of the whole space divert from each other. Note how the elasticity of ROI 2 (soft area) is not only is lower than that of ROI 1 and the median but it decreases with time. This suggests that syneresis decreases the elasticity of the blood clot adjacent to the liquid. Callé *et al* reported a similar findings caused by the ejection of liquid from the clot, where by using high frequency ultrasound there were able to measure an inflection in the speed of the ultrasound wave. This inflection did not occur in all their experiments and often happened at different times [9].

Future work will be aim to characterization of the shear viscosity, since 2D maps of this parameter might bring a good insight in the evolution of the clot mechanical properties and might help in the prediction of rupture of these structures.

5 Conclusion

The work presented here describes a technique for mapping the shear elasticity of the clotting process in two dimensions. By generating shear waves at 3 different locations we were able to characterize the elasticity of 30 x 50 mm^2 2D plane. This proved to be very advantageous since during the coagulation process the expulsion of the free serum (syneresis) seems to affect the mechanical properties of the clot. Analysis of this phenomenon revealed softening and detachment of the thrombus from the walls of the beaker. This suggests that the 2D map has potential in studying and predicting the rupture of clots which is the main concern in deep venous thrombosis resulting in pulmonary embolism.

Acknowledgments

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